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Magnetic resonance spectroscopy – state of art and future

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Summary

Magnetic Resonance Spectroscopy (MRS) has become one of the most important imaging techniques of CNS recently thanks to the significant information concerning chemical compounds, which are produced in the metabolic processes in normal and pathological tissue. The techniques used most often in clinical practice are proton spectroscopy (¹H MRS) and phosphor spectroscopy (³¹P MRS) as those elements play the crucial role in metabolic turn over [1–4]. The first gives information about various metabolic disorders (acethylaspartate acid – nervous cells marker, creatinin – related to metabolic changes, choline –element of cell membranes, lactates – markers of anaerobic metabolism). The second enables evaluation of energetic condition of the cells (phosphate compounds, mono- and biphosphate, phosphocreatinin, adenosine triphosphate ATP). Last years show tendency to quantitative method in spectroscopy. One of the solution seems to be LCMoel, considered in this paper.

Authors describe the basic techniques that are used in in vivo H MRS, P-MRS, C-MRS and possible applications of fMRSI technique as well as hyperpolarized C13.

Key words: magnetic resonance spectroscopy • MRS • hyperpolarized 13C • multi-voxel • multi-nuclei • fMRSI • LCMoel

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Background

Magnetic Resonance Spectroscopy (MRS) has become one of the most important imaging techniques of CNS over 10 years, mainly because this method is enables noninvasive measurement of metabolites which play a central role in cellular metabolism. The techniques used most often in clinical practice, are proton spectroscopy (¹H MRS) [1–4] and phosphor spectroscopy (³¹P MRS) [5–8] – those nuclei play the crucial role in metabolic turn over. The first gives information about various metabolic disorders (acethylaspartate acid – nervous cells marker, creatinin – related to metabolic changes, choline – element of cell membranes, lactates – markers of anaerobic metabolism). The second enables evaluation of energetic condition of the cells (phosphate compounds, mono- and biphosphate, phosphocreatinin, adenosine triphosphate ATP).

Magnetic resonance spectroscopy is based on the phenomenon called “chemical shift”. The resonance frequency of a certain atomic nucleus is to a small (but measurable) degree changed by the surrounding chemical bonds and owing to that the identification of particular molecules is possible.

The features of MRS spectrum (profile), such as the number of resonance lines, their position, the volume under each of them, depend on chemical structure of the examined compound. Analysis of spectrum can lead to identifying the compounds and their proportions in the studied sample. It enables studying simple and complex chemical compounds, as well as the metabolism of living cells in physiologic and pathologic states. The description of spectrum usually requires using a standard substance (internal substance). In the *in vivo* MRS examinations the role is played by phosphocreatinin, while in the *in vitro* – by chemical

compound which gives one sharp intensive signal that is to small extend dependent on the solvent, temperature and concentration (tetramethylsilane TMS in H-MRS, 85% aqueous solution of phosphoric acid in ^{31}P MRS).

Single Voxel Spectroscopy

The practical use of *in vivo* MRS requires a method, which enables registering the spectrum of selected volume, called the "volume of interest" (VOI) or voxel. The localization method, described as Single Voxel Spectroscopy (SVS), is based on modification of the magnetic field in presence of spatial gradient fields, which alter the intensity of constant field depending on the location of selected area. It allows assigning a different resonance frequency to every examined area and acquisition of spectrum from the selected voxel (fig. 1a). Among the advantages are: high homogeneity of the field of the examined volume, easy selective water-suppression, high signal-noise ratio (S/N), and relatively short duration of the examination (around 4-8 minutes). The SVS methods include i.a.: ISIS (*Image-Selected In-Vivo Spectroscopy*), STEAM (*Stimulated Echo Acquisition Mode*), PRESS (*Point-Resolved Localized Spectroscopy*).

The ISIS method generates a sequence of three pulses of 180° followed by a 90° pulse, after which the FID signal is recorded (fig. 1b). Owing to the fact that magnetization (before the 90° pulse) in this technique is directed along the axis the T2 effects are relatively small. The fact that some metabolites (e.g. ATP) have short T2 times makes it possible to use the method in phosphorous spectroscopy. Unfortunately, ISIS cannot be used for localized shimming, as eight acquisitions are necessary to obtain spatial localization.

In proton spectroscopy we usually use the PRESS technique. It is a sequence of 3 pulses: 90° - 180° - 180° . The 90° pulse is followed by two pulses which generate a spin echo from the selected layer (fig. 1c). A double spin-echo method enables good localization and strong suppression of signals outside the selected voxel, while long duration of echo ensures a better visualization of metabolites with long relaxation times.

STEAM (also known as VEST or VOSY) is a technique similar to PRESS. STEAM generates a sequence of three 90° pulses (fig. 1d). It allows shorter echo duration and bet-

ter water-suppression, owing to which the observation of more metabolites becomes possible. The disadvantages of this method include 50% signal loss, higher movement and diffusion sensitivity compared to PRESS [10].

Multi-voxel spectroscopy

Apart from the aforementioned single voxel methods, also known as localized spectroscopy, the CSI (Chemical Shift Imaging) technique, also referred to as MRSI (Magnetic Resonance Spectroscopy Imaging), is used as well.

The CSI technique, which visualizes the chemical shift in one measurement period, it collects spectroscopic signals from multiple small voxels located within a large examined area. The registered signals form a map of spatial distribution of the metabolites (fig. 2c). The signals from particular voxels can be shown as spectra and further analyzed, like in the SVS (fig 2b, 2a).

Spatial CSI coding employs phase-encoding gradients which are out of action while receiving the magnetic resonance signal in order to collect the spectroscopic data (fig. 2c). The major disadvantage is that the shape of individual voxels is less well defined than in single voxel techniques, and the necessity of long echo-durations limits the amount of received data – in case of brain – to 3 bands of metabolites. CSI is also more sensitive to heterogeneity of the magnetic field and to fat tissue-derived artifacts [12].

Long time necessary for acquisition of the CSI data limits the use of this method in clinical practice. Therefore, more and more efforts are put to develop fast spectroscopic imaging techniques, such as Turbo Spectroscopic Imaging (TSI), which is based on acquisition of three echoes, each with a different encoding gradient. The advantage of this procedure is reduction of examination time, unfortunately, at the cost of worsening the S/N because of reduction of T2 signal in subsequent echoes.

There are also techniques which use echoplanar (EP-MRSI) or spiral (spiral MRSI) gradient reading, instead of traditional encoding (fig. 3a, 3b).

In EPSI the oscillating reading gradient is used for acquisition of data. Spatial localization in third dimension can be performed with the use of conventional phase-encoding gradient.

Although the EPSI is one of the fastest MRSI sequences, it is not free from technical problems.

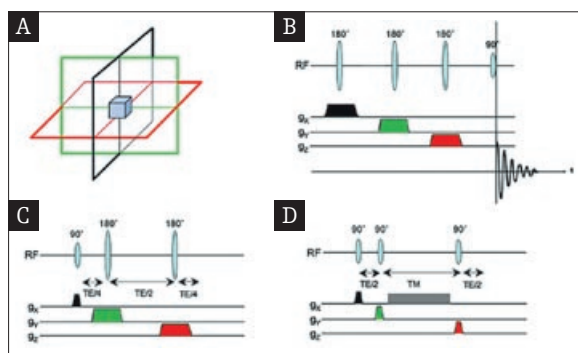


Figure 1. SVS techniques: spatial localization (A), sequence of excitation pulses and gradients in: ISIS (B), PRESS (C), STEAM (D) [9].

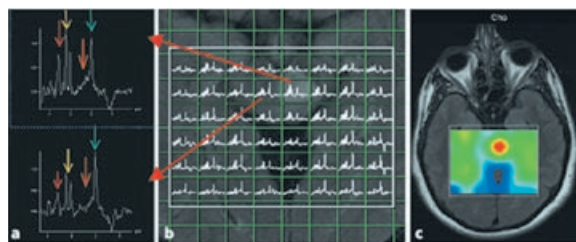


Figure 2. CSI technique as: a map of spatial distribution of choline (c), spectral map (b), spectra from red- marked voxels (a) [11].

EPSI depends on the system of gradients; the oscillating reading gradient, as well as lack of balance in positive and negative lobes can lead to artifacts in metabolites image. This problem can be solved with separate processing of positive and negative lobes, and then joining the results obtained separately. However, such method reduces an already small bandwidth of the spectrum. Compared to other techniques EPSI is sometimes characterized by lower SNR due to short scanning time. Therefore, the scans are repeated two or more times in order to improve the SNR.

Spiral MRSI is a technique similar to EPSI with the difference being that during data acquisition two oscillating gradients are used.

This technique has a few useful features, including ability to control the point-spread function (PSF), time of scanning and SNR. Like in EPSI, in this technique the proportion of SNR depends on the gradient system. Calibration of the system is necessary for minimizing artifacts, in spite of the fact that this technique is less gradient-dependent than the EPI. Spiral MRSI has several advantages in comparison to EPI, but its rare clinical use is probably related to the lack of common availability and advanced reconstruction software.

Another approach to enable reduction of scanning time is simultaneous spectroscopic imaging with the use of SENSE. The method is based on the use of heterogeneity of B1 field of the coil to encode some of the spatial data and, as a result, the number of coding steps and scanning time are reduced.

The key advantage of the method is the fact, that it has no parametric limitations and gives the possibility of combination with other fast spectroscopic techniques. Limited sensitivity seems to be the greatest problem so far, but improvement is expected with the increase of magnetic field [9].

Processing resonance signals

In order to the fact that the FID signal contains a lot of noises, unwanted signals and deformations connected with acquisition, application of various corrective techniques is inevitable. The preprocessing of the resonance signals is conducted in time domain (before the Fourier transformation). They include: offset correction (subtraction of direct electric current produced as a result of interaction between electronic systems when the FID decayed to zero), eddy current correction, zero filling (the signal is extrapolated by appending zero points to improve the resolution of spectrum) and apodization (multiplying the signal with appropriate S/N corrective functions). In medical applications such procedures are usually carried out with the use of software supplied by the tomography producer.

The next stage of processing the resonance spectrum, after the Fourier transformation (in frequency domain – FD), is based on the phase and baseline correction, and on calculating the area below the resonance bands of certain substances.

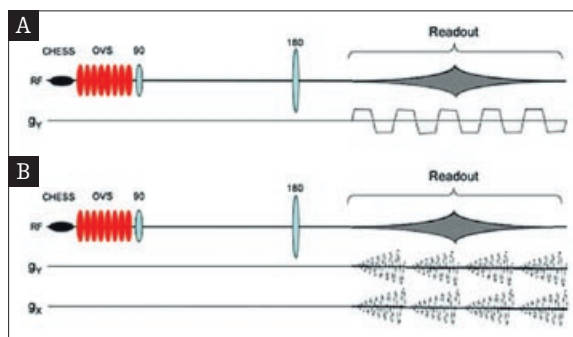


Figure 3. Sequences: (A) proton EPSI-PEPSI, (B) spiral MRSI [9].

During the registration the FID signal is subject to phase shifting which results from equipment settings (the difference between frequencies of transmitter and receiver) and interval between emission of the excitation pulse and registration of the signal. As a result, the NMR spectrum after Fourier transformation is a mix of absorption and dispersion elements (although the information about the concentration of the substance generating resonance band is enclosed in the absorption signal). Phase correction is necessary before the approximation in order to obtain pure absorption spectrum.

Another problem concerning the analysis of spectra is the baseline correction. In general, the baseline is not flat in most *in vivo* spectra, and contains elements far wider than the signals of metabolites. They originate from big, less mobile molecules or equipment artifacts. Proper subtraction of these wide elements is crucial for narrowing the metabolite signals.

All of the newest devices give the possibility to determine the area of spectral peak by calculating its integral value. This is the oldest, but still the fastest and straightforward method to quantify the spectrum lines. Most important, it is lineshape independent. However, this method is prone to many mistakes and its use is limited to well-resolved peaks with good S/N ratio on a flat baseline.

Even the partial overlapping of adjacent lines causes mistakes which can be reduced by Gauss line conversion.

Accurate quantification of the *in vivo* spectra requires mathematical fit of the observed spectrum in order to disentangle the overlapping lines and to reliably estimate their area. Proper phase and baseline correction is the preliminary condition for correctness of this analysis. FD fitting algorithms describe the spectrum as a superposition of lines which correspond to a known model function (Lorentz, Gauss or mix).

Calculation of molar concentrations of the substances on the basis of integrals of corresponding resonance lines requires calibration. In order to that, during the *in vitro* examinations a large amount of standard substance is added to the examined sample (internal standard) or the standard is placed in a separate capillary beside the studied sample (external standard). However, the aforementioned methods of calibration meet serious troubles in the *in vivo* MR spectroscopy; application of the external standard examined separately requires correction for different degree of amplification in

the receiver coil (dependent on the mass of the examined object), while placing the external standard together with the examined patient within the limits of receiver coil causes additional heterogeneity of the magnetic field [13].

In view of the problems with reliable calculations of molar concentrations, the quantitative MRS results are often presented as intensity quotients of resonance signals of particular metabolites (Metabolite Ratios, MR). Nevertheless, the MR must be interpreted with caution as, despite the fact that the metabolites proportion is enclosed within certain range, it does not mean that particular metabolites concentration has changed [14].

One of the programs enabling calculation of total metabolites concentration is LCModel (Linear Combination of Model spectra). It matches linear combination of signals from single metabolites with spectrum of compounds measured independently (fig. 4). Identical measurement technique applied to acquisition of spectrum from the examined object and to the standard is the condition necessary for concentration calculation [15]. LCModel is especially useful for short TE (time of echo) and high concentration metabolites – myoinositol, glutamine.

Flexibility of LCModel is gained owing to the application of base parameters. In practice, it results in the lack of limitations concerning types of metabolites, size of the field, localization methods and sequence parameters [16, 17, 18]. To obtain and match the base parameters is time-consuming and expensive and they can contain experimental mistakes. Therefore, many producers introduce ready-to-use basic parameters for standard protocols of their MRS scanners. The use of LCModel with imported basic settings could support exchange of the results between various research institutions [19].

Owing to the fact, that LCModel is fully automatic it allows obtaining most probable metabolites concentrations and their uncertainties. On the other hand, the lack of subjective interactions helps to exchange and compare the results. The advantage of this program is the repeatability of the result, facility of use, while its application significantly accelerates data processing and eliminates the range of subjective errors.

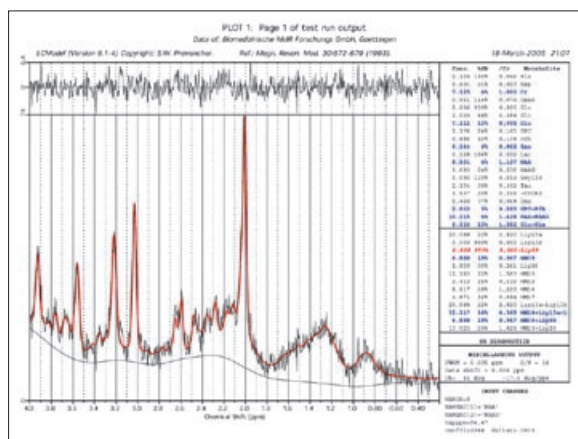


Figure 4. Exemplary test page in LCModel [20].

The disadvantages include unclear documentation, difficulties with accessibility to the source code (the reason being commerciality of the program) and time-consuming manual adjustment.

Functional Spectroscopy (fMRS)

The development of fast imaging techniques in spectroscopy enabled observation of dynamic metabolic processes in brain. The fMRS scanning requires the same equipment as fMRI but uses a different kind of software made for recording various levels of chemical substances. Like fMRI, the fMRS is used for detection of brain signal with the use of RF detector inside the magnet. The main difference between the aforementioned techniques is that fMRS measures the changes of metabolites, while the fMRI – blood oxygenation (BOLD).

One of the substances that can be measured by fMRS is the lactate, considered as determinant of anaerobic metabolism. The lactate is also a by-product of glucose metabolism at the time of brain activity. During visual, auditory and cognitive tasks the level of lactate (in healthy people) increases. The idea of observing the lactate in fMRS is based on the following mechanism: increased electric activity of region of the brain (caused by a certain task) uses up the energy, what leads to the growth of nutrients consumption (i.a. oxygen, glucose) and increase of blood flow. Metabolism is increased and so is the lactate (which is a by-product).

A technique used for fMRS is the previously mentioned PEPSI (proton EPSI).

The disadvantage of this method is a relatively long measuring time and actually small lactate signal, what makes the optimization of spatial resolution still a challenge, if the SNR is to be maintained at the a sufficient level [21].

Multinuclear spectroscopy

The MR spectroscopy uses spectra of various elements. Apart from the aforementioned hydrogen spectroscopy (^1H MRS) and phosphoric spectroscopy (^{31}P MRS), the carbon (^{13}C MRS) and fluorine (^{19}F MRS) spectroscopy are also used [22].

Compared to the ^1H other nuclei, especially ^{31}P and ^{13}C , are less sensitive. Nuclid spectra with appropriate S/N require a much longer measuring time.

While the hydrogen spectroscopy is performed with standard scanners equipped with typical RF coils, the carbon, phosphor and fluorine spectra need additional device/tool for detection and transmission, which operates on resonance frequency of nuclei of our interest. A typical spectrum of the aforementioned nuclids is complex; it has a wide band of chemical shift and contains signals which are split into multiplets. The splitting is caused by nuclei-proton coupling and can only be removed by decoupling. One of the ways to enhance the signal is to decouple the spins; it consists in the use of double resonance which eliminates multiplet splitting and makes their elements merge into one stronger signal. At a given induction of field, which causes resonance of nuclei, it is also affected with the use

of electromagnetic wave of frequency, which saturates the protons coupled with nuclids. This kind of frequency results in equalizing the multiplexing.

Enhancement of the signal of amplitude can also be obtained by the effect of double nuclear resonance – Nuclear Overhauser Enhancement (NOE). It consists in saturation of the coupled protons while inducing the nuclids, and detection of their signal [23].

Hyperpolarized ^{13}C

The main reason for general low sensitivity of NMR techniques is low polarization of nuclei in thermal balance; even in the high fields only one in 10^5 of nuclei contributes to the measured signal. As for ^1H , low sensitivity is compensated by high concentration of protons in the tissues. Very low MR sensitivity to the ^{13}C carbon compared to H is a consequence of gyromagnetic factor and low abundance. However, it is possible to improve polarization of selected nuclei by a factor of 100000 or more. The enhancement of polarization can be obtained by increasing the magnetic field. Another idea to improve the polarization is to create artificial non-equilibrium arrangement of the nuclei. The state of hyperpolarization, in which the difference in population of spins of high or low energy increases by a few rows compared to the balance state and does not depend on the outer magnetic field. Hyperpolarization concerns mainly the organic substances containing ^{13}C or the following nuclei: ^3He , ^{129}Xe and ^{15}N .

There are two methods of hyperpolarization: DNP (dynamic nuclear polarization) and PHIP (para-hydrogen-induced polarization). As for the first, high polarization of electron spins is relocated onto coupled nuclear spins. In the Ardenkjaer-Larsen et al [24] method the material which contains ^{13}C is admixed with a substance containing unpaired electrons. Microwave radiation similar to resonance of electrons is used for moving the polarization from electrons to carbon nuclei. The pumping process can increase the nuclear polarization to 20–40%.

On the other hand, in the PHIP method nuclear polarization is increased by chemical reaction with parahydrogen [25, 26]. The molecular spin of parahydrogen can be exchanged for ^{13}C nuclear polarization by means of either diabatic field cycling or by a sequence of RF pulses.

Indicators enriched with polarized ^{13}C are used for visualizing metabolic processes in C NMR spectroscopy. Injection of labeled ^{13}C glucose or acetate enables discovery of unknown disorders in NAA synthesis, tricarboxylic acids cycle and glycolysis [27, 28]. Hyperpolarization enlarges signal amplitude what results in reduction of scanning time to a few seconds.

It ought to be mentioned that hyperpolarization samples are characterized by non-thermal polarization and such hyperpolarization is performed *ex situ*. The fact that the nuclear polarization does not disappear after dissolution in liquid is typical for the samples [29].

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